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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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| Applicant | : Hsu Ching-Hsiang et al. | Art Unit | : 1632 |
| Serial No. | : 09/778,672 | Examiner | : Q. Janice Li |
| Filed | : February 7, 2001 | | |
| Title | : LIVE VACCINES FOR ALLERGY TREATMENT | | |

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

## DECLARATION UNDER 37 C.F.R. §1.132 OF DR. CHING-HSIANG HSU

I, Ching-Hsiang Hsu, a citizen of Taiwan, residing in Taibao City, Taiwan, hereby declare as follows:

### Introductory Remarks

1. I received a Medical Degree from the Chinese Medical College, Taiwan in 1988 and a Ph.D. in Immunology from Graduate Institutes of Immunology & Microbiology at the National Taiwan University in 1996. As a physician, I have board certifications as a specialist in pediatrics, neonatology, emergency medicine, and allergy and clinical immunology. I was an intern and resident at Mackay Memorial Hospital, Taipei (1987-1993) and an attending physician in pediatrics at St. Paul Hospital, Taipei. From 1996-2001, I was Director of the Pediatric Department at the God's Help Hospital, Taipei. Since 1997, I have been an associate professor at the Chinese Medical Institute. Since 1999, I have been a founder, President, and Chief Executive Officer of GenMont Biotech, Inc., a Taiwanese company that is the assignee of the above-referenced patent application. I am a member of a number of professional societies, and an author on numerous scientific publications as detailed in the attached resume (Appendix A).

CERTIFICATE OF MAILING BY FIRST CLASS MAIL

I hereby certify under 37 CFR §1.8(a) that this correspondence is being deposited with the United States Postal Service as first class mail with sufficient postage on the date indicated below and is addressed to the Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

July 9, 2003
Date of Deposit

Megan O'Meara
Signature

Megan O'Meara
Typed or Printed Name of Person Signing Certificate

2. I am the inventor of the subject matter claimed in the above-referenced patent application, and I have read and understand its contents.

3. I have been advised and understand that the Examiner has rejected claims 24-33, 35-39, and 41-49 of the above-referenced application in view of U.S. 5,958,891 ("the '891 patent," herein), and Medaglini (1995) *Proc. Natl. Acad. Sci. USA* 92:6868 ("Medaglini", herein).

**General Description of the Claimed Invention**

4. I understand and have been advised that claims 24-33, 35-39, and 41-49 of the above-referenced application are at issue. These claims, as presently amended, relate to methods of administering, to a subject, a bacterium that can express a protein allergen. We discovered that a bacterium that expresses a protein allergen can be used to induce immunological tolerance towards the protein allergen. Claims 24-33, 35-39, 41-43, 46, 48, and 49 relate to methods of suppressing IgE production.

5. The claimed methods require that the allergen is expressed in the bacterium while the bacterium is in the subject. I emphasize that the allergen is produced by the bacterium using prokaryotic transcription and translation machinery. This mode of allergen production -- prokaryotic production -- is the mode taught by the specification of this application. Thus, page 1, lines 13-15, states:

> The invention is based on the discovery that recombinant lactic acid bacteria expressing a protein allergen can induce immunological tolerance against the allergen in animal models. [emphasis added]

Page 3, lines 3-7, is to similar effect:

> [T]he skilled artisan can clone into a bacterial expression vector a nucleotide sequence encoding the allergen. This expression vector is then introduced into a lactic acid bacterium, which in turn is administered to an individual to ameliorate or prevent a subsequent symptom . . . characteristic of an allergy. [emphasis added]

Neither the specification nor the claims purport to include expressing a nucleic acid encoding an allergen in eukaryotic cells. Thus, both the claims and the specification require prokaryotic production of allergens, not eukaryotic production.

**Eukaryotic and Prokaryotic Expression**

6. Prokaryotic and eukaryotic cells are vastly different. First, the mechanisms of prokaryotic and eukaryotic transcription and translation differ substantially. For example, promoters, secretory sequences, and ribosomal binding sites can differ between prokaryotic and eukaryotic systems. A typical prokaryotic promoter includes a –10 and –35 element that is recognized by prokaryotic RNA polymerase. In contrast, eukaryotic RNA polymerase II typically interacts with the TBP complex which can bind to the TATA box. Eukaryotic transcription is also regulated by enhancer sequences, activators, repressors, and nucleosomes. These elements are either absent from prokaryotic cells or differ mechanistically.

7. The CMV promoter from cytomegalovirus (CMV) is a powerful promoter in eukaryotic cells. A skilled artisan might link a coding sequence to the CMV promoter to express the coding sequence in a eukaryotic cell. However, I am not aware of any suggestion that such a construct would be expressed in a prokaryotic cell so that the coding sequence would be translated in a prokaryotic cell.

8. A second critical difference between an antigen produced by a eukaryotic cell and an antigen produced by a prokaryotic cell is that the immune system distinguishes between the two types of antigen production. Eukaryotic cells process proteins that are produced intracellularly into peptides. These peptides are displayed on class I MHC (multi-histocompatibility complex) proteins on the cell surface. Proteins translated within prokaryotic cells are not displayed on class I MHC proteins. An antigen produced by an prokaryotic cell may be processed by a eukaryotic cell and displayed on the cell surface in a complex with class II MHC proteins. Presentation by class I and class II differs. For example, class I presentation is seen by $CD8^+$ T cells, whereas class II presentation is seen by $CD4^+$ T cells. Thus, an exogenous

protein produced by a prokaryotic cell in a subject is viewed by the subject's immune system in a very different context from an exogenous protein produced by an eukaryotic cell of the subject.

## Allergies and the Immune Response

9. Allergic diseases are typically caused by a hypersensitivity to an exogenous antigen, termed an "allergen." A main characteristic of allergic diathesis is development of a sustained immunoglobulin E (IgE) response to one or more allergens. IgE production is frequently highly dependent on IL-4 (interleukin 4) and strongly inhibited by IFN-γ (interferon gamma).

## U.S. 5,958,891

10. I have read and understood U.S. 5,958,891, which the Examiner refers to as Hsu *et al.* Further, I am an inventor on the '891 patent and, accordingly, am particularly intimate with the scientific content of this patent. The result of the method described in the '891 patent is suppression of IgE immunoglobulin production.

11. The '891 patent involved, in part, the recognition that $CD8^+$ T cells could be used to suppress IgE synthesis, for example, by stimulating IFN-γ production. The '891 patent describes a method of producing peptides that are displayed on MHC class I molecules so that these peptides would be presented to $CD8^+$ T cells. To display peptides from allergens on MHC class I molecules, the allergens were expressed in eukaryotic cells of the host. In one example, nucleic acid encoding the allergen was injected intramuscularly so that the eukaryotic cells of the subject would produce and process the allergen into peptides for display on MHC class I molecules. Indeed, we found and described in the '891 patent that, in a particular implementation, this mode of allergen display caused enhanced IFN-γ production and that this response depended on $CD8^+$ cells:

> Unfractionated splenocytes from DNA immunized mice secreted high levels of IFN-r [sic] in response to specific [antigen], and this response was markedly reduced by depletion of CD8+, but not CD4+ cells.

(column 9, lines 3-6 of the '891 patent).

12. Thus, the '891 patent discusses delivering a nucleic acid encoding an allergen directly into eukaryotic cells using a pharmaceutical composition containing a recombinant plasmid and a pharmaceutically acceptable carrier. The described methods for delivering the recombinant plasmid include "intramuscular injection, intranasal delivery or intratracheal delivery."

**Medaglini**

13. I have read and understood the Medaglini reference. The goal of Medaglini is to stimulate immunoglobulin production at mucosal surfaces. The mucosa are the first line of defense against many pathogens. Thus, the goal of Medaglini's method is to elicit an enhanced immunoglobulin response against pathogens:

> We have developed a system whereby non-pathogenic Gram-positive commensal bacteria that occupy a specific mucosal niche may be used to stimulate a mucosal immune response against a pathogen that enters the mammalian host at a specific site (oral, intestinal, or vaginal). [emphasis added]

(page 6868, column 1 of Medaglini)

14. The reasoning of Medaglini is that antigens presented by the *Streptococcus gordonii* bacteria are presented in a mode that resembles a foreign antigen of a pathogen attacking the mucosa:

> Because most human pathogens enter the host through the mucosa, protection from such pathogens may be maximized by employing vaccine strategies that induce an immune response at the site of infection. . . . [S]ince *S. gordonni* is a human commensal, we expect that in humans the colonization would be prolonged, resulting in a sustained immune response. [emphasis added]

(page 6872, column 1 of Medaglini)

Thus, Medaglini takes advantage of prokaryotic expression in *Streptococcus gordonii* to mimic a pathogen invading the mucosa. The result is stimulation of the humoral immune response and enhanced immunoglobulin production.

15. Whereas Medaglini teaches stimulation of immunoglobulin production, Medaglini does not teach or suggest suppression of immunoglobulin production. In particular, Medaglini does not teach or suggest how a human commensal can be used to suppress IgE production. Nor does Medaglini suggest the radical position that *Streptococcus gordonii* could be used to delivery a nucleic acid encoding an antigen from the *S. gordonii* bacterium into a eukaryotic cell of a subject so that the eukaryotic cell would express the nucleic acid and produce the antigen.

**The Examiner's Rejection**

16. I have also been advised and understand that the Examiner has rejected claims 24-33, 35-39, and 41-49 of the above-referenced application. I have been advised and understand that the rejection of at least claims 24 and 43 is based, in part, on the following assertion:

> Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to combine the teachings of Hsu et al. and Medaglini et al. by using the delivery vehicle provided by Medaglini et al. in the delivery of the Der p5 with a reasonable expectation of success. The ordinary skilled artisan would have been motivated to modify the claimed invention because the non-pathogenic bacterial system could express the allergen stably, and using oral delivery route is less painful for patients. Thus, the claimed invention as a whole was prima facie obvious in the absence of evidence to the contrary.

(page 6 of the Office Action dated April 9, 2003)

I address below (1) why there is no motivation to combine Medaglini and the '891 patent, and (2) why there is no expectation for success for the alleged combination.

**No Motivation to Combine**

17. The Examiner suggests that there is a motivation to combine because Medaglini provides a "nucleic acid delivery vehicle" to substitute for the intramuscular injection embodiment of the '891 patent. As I understand it, the alleged motivation is that the Gram-

positive commensal bacteria of Medaglini could be used to transfer nucleic acid from the bacterium into a eukaryotic cell so that the nucleic acid can be expressed in the eukaryotic cell as taught by the '891 patent. However, Medaglini does not teach that nonpathogenic Gram-positive commensal bacteria can be used to deliver a nucleic acid from the bacteria into a eukaryotic cell. For example, Medaglini states at page 6870, column 2:

> Recombinant plasmid pSMB70 was used to transform competent *S. gordonii* GP251 cells. Erythromycin-resistant transformants were tested for both the loss of chloramphenicol resistance and expression of M6 protein. Genetic analysis showed that 100% of erythromycin-resistant, chloramphenicol sensitive transformants expressed the M6-Ag5.2 fusion protein on the cell surface. One such transformant was chosen as representative and named DM100. * * * Recombinant strain DM100 exhibited positive fluorescence when reacted with [a] Ag5.2 specific monoclonal antibody. [emphasis added]

Nor, I am currently aware of any evidence that a nonpathogenic Gram-positive commensal bacterium can be used to deliver a nucleic acid from the bacterium into a mammalian cell. Because Medaglini does not teach a nucleic acid delivery vehicle for introducing nucleic acids into eukaryotic cells, I respectfully submit that the Examiner's suggestion that one skilled in the art would be motivated to combine Medaglini and the '891 is unfounded.

18. Moreover, Medaglini and the '891 patent include very contrasting teachings. Some differences are summarized in the following table.

| | Medaglini | '891 |
|---|---|---|
| *Agent* | Commensal bacterium | Injected Nucleic acid |
| *Mode of Expression* | Prokaryotic | Eukaryotic |
| *Mode of Ag Display* | [not discussed] | Class I MHC |
| *Result* | Enhanced immunoglobulin production | Suppressed immunoglobulin (IgE) production |
| *Proposed Mechanism* | Agent stimulates Mucosal Immune Response | MHC presented antigens induce CD8+ T cells production of IFN-$\gamma$ to suppress immunoglobulin production |

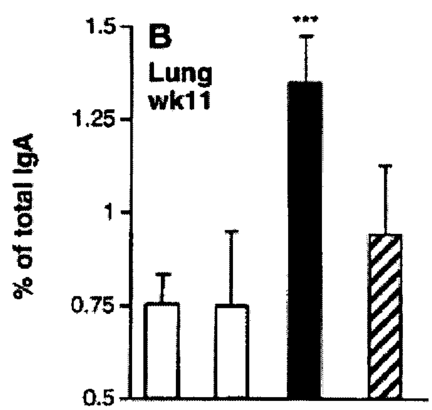
The five criteria in the table above are central features of both references. At least with respect to these five criteria, there is no common ground between the two references.

19. Indeed, the two references teach away from each other. First, one skilled in the art, seeking to suppress immunoglobulin production would not turn to a system that enhances immunoglobulin production. Second, one seeking to exploit the teachings of the '891 patent, which describes presenting antigens on class I MHC molecules after antigen expression in eukaryotic cells, would not turn to a prokaryotic expression system. Prokaryotic expression is not known to result in antigen presentation on class I MHC molecules since typically production of the antigen in a eukaryotic cell is required for presentation on class I MHC molecules.

## No Expectation of Success

20. Further, there is no expectation of success for the combination proposed by the Examiner. Evidence presented by both Medaglini and the '891 patent opposes the outcome advanced by the Examiner.

21. The evidence in Medaglini is that an antigen expressed in a Gram-positive commensal bacteria causes a stimulation of immunoglobulin production. FIG. 4B of Medaglini shows:




The above figure shows the secretory IgA response to the exogenous antigen Ag5.2 in lung lavages. The effect of live DM100 bacteria which express the exogenous antigen produces the result shown by the black bar (third from left), an effect that exceeds the result of the control

shown in the first bar (on the left). Medaglini interprets this data as follows on page 6871, column 1:

> At week 11, significant levels of Ag5.2 IgA were detected in lung lavages of mice colonized with DM100 compared to mice colonized with wild-type GP204 (Fig. 4B).

Other data in Medaglini are to similar effect. Medaglini summarizes in the Abstract:

> A <u>significant increase</u> of Ag5.2 specific IgA with relation to the total IgA was detected in saliva and lung lavages when compared with mice colonized with wild-type S. gordonii. [emphasis added]

Thus, the evidence in Medaglini shows that a Gram positive commensal expressing an antigen <u>increases</u> immunoglobulin response to the antigen. This evidence is contrary to outcome alleged by the Examiner since it is contrary to the proposition that a Gram positive commensal expressing an antigen would <u>reduce</u> immunoglobulin response to the antigen.

22. Similarly, the evidence presented in the '891 patent opposes the outcome advanced by the Examiner. The '891 patent includes data that shows that $CD8^+$ cells mediated immunoglobulin suppression. For example, when $CD8^+$ cells from mice injected with a nucleic acid encoding the Der p 5 allergen were transferred to a naïve mouse, IgE production was inhibited in the naïve mouse:

> Mice were intramuscularly injected with 100 .mu.g of pCMV or pCMVD, and sensitized with Der p 5 or saline 3 weeks later. Three weeks after sensitization, mice received inhalation challenge with Der p 5 or saline. 18 hrs after inhalation, pulmonary resistance was determined. Unfractionated, $CD8^-$-depleted, or $CD4^+$ -depleted splenocytes were adoptively transferred to naive recipients. The recipients were then challenged with Der p 5 and alum adjuvant, and Der p 5-specific IgG2a and IgE responses were determined. Both $CD4^-$ cells and the unfractionated group showed significant inhibition of Der p 5-specific IgE production. In contrast, the $CD8^-$ group demonstrated no inhibitory effects, indicating that $CD8^+$ T cells could down-regulate the ongoing production of IgE (FIG. 3B).

(column 8, lines 44-58 of the '891 patent)

The '891 patent also notes the $CD8^+$ mediated effects require antigen presentation on Class I MHC molecules. Antigens are presented on class I MHC molecules when they are expressed within eukaryotic cells:

> [I]t is well established that peptides derived from intracellular Ags are generally presented to $CD8^+$ T cells by major histocompatibility complex (MHC) class I molecules, which are expressed on virtually all somatic cells, while peptides derived from extracellular Ags are presented to $CD4^+$ T cells by MHC class II molecules normally expressed by specialized, Ag-presenting cells.

(column 2, lines 7-13 of the '891 patent, references omitted)

Thus, the evidence in the '891 patent indicates the immunoglobulin levels can be suppressed by a CD8 mediated pathway and that CD8 cells can be stimulated to suppress immunoglobulin levels by presenting antigens to them on class I MHC molecules when the antigens are expressed within a eukaryotic cell.

23. Because the evidence in the '891 patent suggests that eukaryotic expression of allergens is important to immunoglobulin suppression and the evidence in Medaglini suggests that prokaryotic expression results in immunoglobulin enhancement, there is evidence that suggests that the alleged combination of the '891 patent and Medaglini would fail. This evidence refutes the obviousness rejection made by the Examiner.

24. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title XVIII of the United States Code, and that such willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

Date: Jun 23, 2003

Hsu Ching-Hsiang M.D., Ph.D.
Dr. Ching-Hsiang Hsu

APPENDIX A

# Curriculum Vitae

## Ching-Hsiang Hsu (□□□) O.M.D., M.D., Ph. D.

### PERSONNAL DATA:

**Postal Address**: No.8, Nan-Ke 7th. Rd., Tainan Science-Based Industrial Park,
Tainan County, Taiwan, R.O.C

**Telephone No**:+886-6-505-2151

**Fax No**: +886-6-505-2152

**Birth date/Place**: Dec 4, 1961/Taipei, Taiwan

**Sex**: Male

**Nationality**: Taiwan

### EDUCATION:

September 1981- June 1988 Chinese Medical College, Taiwan
B.S in Medicine

September 1993- June 1996 Graduate Institutes of Immunology & Microbiology,
National Taiwan University
Ph. D. in Immunology

### PROFESSIONAL EXPERIENCE:

June 1987- May 1988 Internship, Mackay Memorial Hospital, Taipei

June 1990-August 1993 Residency, Mackay Memorial Hospital, Taipei

September 1993-May 1996 Attending Physician in Pediatrics, St. Pual Hospital

| | |
|---|---|
| June 1996-Jun 2001 | Director of Pediatric Department, God's Help Hospital |
| June 1997-Present | Associate Professor, Chinese Medicine Institue |
| June 1998-Present | Consultant of Sun-Ten Pharmaceuticals Co., |
| September 1999-Present | Consultant of Phytoceutica Pharmaceutical Co. & East West Pharmaceuticals International LLC |
| | P resident & C.E.O of GenMont Biotech Inc. |

**HONORS AND AWARDS**

1.1992 Mackay Memorial Hospital, Best Resident Award

2. 1993 Chinese Taipei Pediatric Association, Fellowship Research Award

3. 1995 College of Medicine, National Taiwan University, Research Award

4. 1996 Chinese Taipe Pediatric Association, Nestal Research Award

5. 1996 WANG MING-NING MEMORIAL FOUNDATION, Research Award

6. 1997 Chinese Taipei Pediatric Association, The New Categorical Award

**BOARD CERTIFICATION:**

1. Specialist of Pediatrics
2. Specialist of Neonatology
3. Specialist of Emergency Medicine
4. Specialist of Allergy and Clinical Immunology

**PROFESSIONAL ACTIVITIES:**

1. Member, The Society of Pediatrics
2. Member, The Society of Neonatology
3. Member, Infectious Disease Society of the Republic of China
4. Member, The Society of Ultrasound in Medicine
5. Member, The Formosan Medical Association

**Summary of Research Experience**

1. Establishment of an animal model for the study of atopic dermatitis

   I and my colleagues establish first animal model for atopic dermatitis induced by allergen.

2. Prevention and treatment of allergic disease by direct allergen-gene transfer

   I and my colleagues has successfully demonstrated that direct gene transfer (gene therapy) could treat and prevent IgE-mediated allergic diseases including atopic dermatitis and airway hyperreactivity. This is a major breakthrough in the treatment of allergic diseases.

3. To investigate the working mechanisms of tradition Chinese medicine in the treatment of allergic diseases.
4. Study the mechanisms of oral tolerance, by transgenic animal and plant

**Invited Symposium Lectures**

1. The Second Asian Pacific Congress of Allergology and Clinical Immunology. Taipei, Taiwan, R.O.C. November 18-22, 1995
2. The 88th Symposium of Formosan Medical Association, Taipei, Taiwan, December.1996
3. 5$^{th}$ Wesdt-Pacific Allergy Symposium,1997

**Major Conference Abstracts**

1. Application of nuclear medicine in diagnosis of fever of unknown origin. 2 cases report. **Hsu CH**, Huang FY, and Shin BF. Acta Paediatrcia Sinica 1992;33:14.

2. Computerized dysmorphology database; preliminary report. **Hsu CH**, Lin SP, Huang FY. Acta Paediatrcia Sinica 1992;33:95.

3. The Wolf-Hirschhorn syndrome; A newborn case report. **Hsu CH**, Lin SP, Hung HY, Shen EY, Yang SY. Acta Paediatrcia Sinica 1992;33:98.

4. Kawasaki Disease; 5 years experience. **Hsu CH**, Chen MR, Huang FY, Kao HA, Hung HY, Hsu CH. Acta Paediatrcia Sinica 1992;33:114.

5. Duplex ultrasound assessment of gut blood flow velocity. **Hsu CH**, Lee HC. Acta Paediatrcia Sinica 1993;34:76.

6. Inhibition of an in vivo allergen-specific IgE response by direct gene transfer. **Hsu CH**, Chua KY and Hsieh KH. Acta Paediatrica Sinica 1996;37:137.

7. Immunoprophylaxis of allergen-induced immunoglobulin E synthesis and airway hyperresponsiveness in vivo by genetic immunization. **Hsu CH**, Chua KY, and Hsieh KH. Acta Paediatrica Sinica 1996;37:136.

**PUBLICATIONS (first author, original articles)**

1. Hsu CH, Lin SP, and Huang FU. Computerized Dysmorphology Database Mackay (CDDM). Acta Paed Sin 1993;35:19-26. MI

2. Hsu CH, Lee HC, Chiu NC, and Huang FY. Duplex Doppler Assessment of Visceral and Cerebral Blood Flow Velocity in Newborns. J Formos med Assoc 1993;92:803-6.MI

3. Hsu CH, Chen MR, Hwang FY, Kao HA, Hung HY, and Hsu CH. Efficacy of plasmin-treated Intravenous Gamma-globulin for therapy of Kawasaki Syndrome. Pediatr Infect Dis J 1993;12:509-12.SCI

4. Hsu CH, Lee HC, Huang FY. Duplex ultrasonographic assessment of gut blood flow velocity. J Ultrasonund Med 1994;13:15-18.SCI

5. Hsu CH, Chua KY, Huang SK, Chiang IP, and Hsieh KH. Development and Characterizations of a murine Dermatitis Model. J Allergy and Clin Immunol 1995;95:806.SCI

6. Hsu CH, Chua KY, Huang SK, Chiang IP, and Hsieh KH Glutathone S-transferas induces murine dermatitis that resembles human allergic dermatitis. Clinical and Experimental Allergy 1996;26:1329-1337.SCI

7. Hsu CH, Chua KY, Huang SK, and Hsieh KH et al. Immunoprophylaxix of Allergen-induced IgE synthesis and Airway hyperresponsiveness by gentic immunization. Nature Medicine 1996;2:540-544.SCI

8. Hsu CH, Chua KY, Huang SK, and Hsieh KH et al. Inhibition of allergen-specific IgE by direct gene transfer. International Immunology 1996;8: 1405-1411.SCI

9. Hsu CH, Shyu YY, and Li MH. The mechanisms of anti-asthmatic formulae in traditional Chinese medicine in the treatment of allergen-induced airway inflammation. J Chin Med 2000;11:111-121

10. High Eosinophil Cationic Protein Level in Asthmatic Patients with "Heat" Zheng Hsu CH et al . Am J Chinese medicine (accepted) 2001. SCI

11. Suppression of allergic airway inflammation by co-administration of interleukin-18 and interleukin-12. Hsu CH er al. J Immunology (submitted) 2001